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Da 37. (amended) The method of claim 20, further comprising adding a blocker probe to the hybridization step, wherein said blocker probe hybridizes to excess non-hybridized capture sequence probes.

REMARKS

Applicants respectfully request favorable reconsideration in view of the herewith presented amendments and remarks.

Claims 1-55 are pending in this application. Claim 47 is withdrawn from consideration. Claims 1-46 and claims 48-55 are rejected.

35 U.S.C. §102

Claims 22-27, 30-36, and 40-45 have been rejected under 35 U.S.C. §102(b) as being anticipated by Snitman, et al. (USPN: 5,641,630). Applicants respectfully disagree with this rejection.

The Examiner contends that Snitman, et al. "teaches that the first probe (capture probe) and second probe (signal probe) *may be* labeled (see column 6, lines 1-67) which indicates that probes could be used with a label or without a label to detect the DNA-RNA hybrid" (Official Action- October 17, 2002; page 3, 1st full paragraph; emphasis added). This section merely describes two probes which each form hybrids with the target and the first probe being bound to a first complexing agent, and the second probe bound to a detectable label. The Snitman method describes a direct detection means using at least one detectably labeled probe. In every embodiment of the Snitman reference, at least one labeled or modified probe for direct detection means is used. No method of detecting the sandwich complex using an unmodified second probe is taught or suggested in this reference. Therefore, at least one probe, according to Snitman, must be labeled or modified and the Examiner's interpretation that the second probe *may be* labeled, is not justified.

The Examiner further contends that since the instant independent claims (i.e., claims 22 and 40) are of the open "comprising" format, any additional steps are permitted in the

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claim. The instant claims specifically recite the use of the unlabeled probe and therefore are not anticipated or rendered obvious in view of the Snitman reference.

It is important to point out that Snitman does not enable the use of an unlabeled probe. The Examiner contends that the Snitman probes could be used without a label, however the hybrid would not be detectable without a labeled probe. There is no mention of detecting the sandwich complex without a labeled probe. Applicants respectfully direct the Examiner's attention to the Brief Summary, col. 4, lns. 45-67 of the Snitman reference. Snitman reports a method of detecting a target nucleic acid sequence from a solution employing two probes, where a "detectable reporter group is attached to the second probe sequence" and an "assay is then performed to detect and quantitate the bound reporter" (col. 4, lns. 56-57, lns. 66-67). Although the Examiner contends that the second probe *may* be unlabeled, applicants assert that the second probe as described and enabled by Snitman must be labeled or modified with a reporter group (col. 6, lines 52-67) otherwise the sandwich complex would be undetectable.

Applicants respectfully direct the Examiner's attention to col. 6, ln. 52 of Snitman which states "A reporter group may be covalently attached to the second probe" followed by examples of different potential reporter groups in lines 53-67. Since other non-covalent labeling methods are provided by Snitman, the reporter group may or may not be *covalently* attached to the second probe. Sandwich hybridization assays as performed by others and described in the Background of Snitman include a "second, labelled nucleic acid probe" (Snitman, Col. 3, lns. 22-39). One skilled in the art would understand that the second probe is labeled, especially in view of the lack of disclosure of using an unlabelled second nucleic acid probes in Snitman.

The Snitman specification also does not describe the use of an antibody which recognizes the RNA:DNA hybrid as a means for detecting the complex as claimed in the present invention. Snitman only describes direct detection of the sandwich complex using labeled probe. This is the only means by which one would detect the sandwich complex using the Snitman invention. Snitman does not even contemplate using an antibody for detecting the complex. Claims 19, 21, 22-31, 33-36, and 42 of the instant invention, however, use indirect detection of the hybrid by using a detectable antibody specific for the hybrid. Applicants respectfully disagree with this rejection, as the cited reference does not mention using a hybrid-specific antibody to detect the hybrid complex. Since Snitman requires a labeled second probe and does not disclose using an antibody for detecting the

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sandwich complex, claims 22-27, 30-36, and 40-45 of the instant application are not anticipated by or rendered obvious over Snitman, et al. Reconsideration and withdrawal of this §102 rejection is respectfully requested in view of applicants' aforementioned arguments.

NEW GROUNDS OF REJECTION

35 U.S.C. §102

Claims 1-5, 10-21, 32, 37-39, and 48 have been rejected under 35 U.S.C. 102(e) as being anticipated by Coull, et al. (USPN 6,110,676). Applicants respectfully disagree with this ground for rejection and with the Examiner's interpretation of the Coull reference.

The Examiner contends that Coull describe a method for detecting a target nucleic acid by hybridizing a single-stranded nucleic acid to two or more probes to form double-stranded hybrids, where one of the probes is labeled and at least one of the other probes is unlabelled, and blocker probes are added to suppress non-specific binding of detectable probes. However, applicants respectfully point out that in contrast to the Coull reference, the instant invention as claimed provides three types of probes: capture sequence probes (CSP), signal sequence probes (SSP), and *blocker probes that hybridize to excess non-hybridized capture sequence probes.*

Example 4 of Coull describes using a blocker probe which hybridizes to non-target nucleic acids as a way of suppressing non-target nucleic acids binding a labeled probe. Coull, et al. describes the use of "unlabeled probes" (col. 27, lines 44-58, col. 35, Ins. 30-50, and col. 36, Ins. 1-50) which the Examiner equates to the blocker probes of the present invention. The use of blocker probes in Coull to suppress the binding to non-target nucleic acid sequences functions by hybridizing to non-target sequences, thereby preventing non-specific binding of the detectable probes to the non-target sequences. In contrast, applicants' blocker probes "hybridize to the excess non-hybridized capture sequence probe" as required by instant claims 1-2, depending claims 4-5, 10-21, 32, 37-39, and 48. The unlabeled blocker probes of Coull bind to non-target nucleic acid sequence. The addition of blocker probes of the instant invention prevents non-hybridized CSPs from hybridizing to cross-reactive nucleic acid sequences present in the target sequence.

As shown in col. 28, Ins. 1-24 of Coull, the labeled and unlabeled probes (blocker probes) are essentially complementary to the target. However, the blocker probes of the

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instant invention do not hybridize to the target nucleic acid sequence because the blocker probe sequence is **IDENTICAL** to the target sequence. Thus, the probes of Coull are not the blocker probes of the instant invention because Coull's probes are complementary to the target and those of the instant invention are identical to them. The blocker probes of Coull differs from the claimed invention and do not anticipate those of the instant invention.

The use of unlabeled probes (or blocker probes) in Coull is illustrated in Example 6. Applicants respectfully direct the Examiner's attention to col. 36, lines 5-9, where Coull describe "PNA and DNA probes which were complementary to each of the two target nucleic acid sequences...The probes were prepared as both labeled with fluorescein (detectable probes) and unlabeled (blocker probes)." Coull example 6 demonstrates that unlabeled probes can be used to suppress the binding of detectable probe to non-target sequence. This function requires that the unlabeled probe be complementary to the target sequence. In contrast, the blocker probes of the claimed methods are not complementary to the target, but rather contain sequence which is **IDENTICAL** to the target nucleic acid. The addition of the claimed blocker probes prevents non-hybridized capture sequence probes from hybridizing to cross-reactive nucleic acid sequences present in the target, thereby increasing the specificity of detection. See Figure 2 of the instant specification. These blocker probes bind excess capture sequence probes. Therefore, Coull, as cited by the Examiner, does not teach or suggest blocker probes as claimed.

Therefore, Coull, as cited by the Examiner, does not disclose blocker probes as described and claimed in the instant specification. Since the claims recite a method of detecting target nucleic acids using blocker probes and Coull does not anticipate the blocker probes as used in the claimed methods, applicants respectfully request reconsideration and withdrawal of this §102 rejection.

35 U.S.C. §103

Claims 6-9, 45-46, 49, and 50-55 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Coull, et al. (USPN: 6,110,676) and in view of Shah, et al. (USPN: 5,629,156). Applicants respectfully disagree with this §103(a) rejection.

The Examiner uses Shah to teach dual capture methods, biotinylated probes on both ends, distance between probes that hybridize to target, and bridge probes. Additionally, the Examiner uses the blocker suppression methods of Coull to reject the instant claims.

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However, it appears that the Examiner has misinterpreted Coull as discussed in detail above. Without Coull, the Shah reference does not satisfy the requirements as claimed in the instant invention. The Coull reference at col. 7, lines 39-67, col. 8, lines 1-67, col. 27, lines 44-58, col. 35, lns. 30-50, and col. 36, lns. 1-50, as cited by the Examiner, relates to the use of "unlabeled probes." The Examiner equates these to the blocker probes of the present invention. This interpretation is, however, incorrect. The blocker probes taught by Coull reduce background noise by hybridizing to non-target sequences, thereby preventing the detectably labeled probe from hybridizing to unintended targets. The blocker probes of the instant invention which bind excess capture sequence probes and therefore do not affect hybridization of the signal sequence probe to target. Thus, the blocker suppression method of Coull is different from that of the instant invention. Coull does not teach or suggest the blocker probes as used in the claimed methods. Shah does not use "bridge probes" to reduce background as the Examiner has suggested. Applicants believe the deficiencies in the Coull, et al. reference discussed above, are not remedied by the Shah reference as relates to claims 6-9, 45-46, 49, and 50-55.

The Examiner contends that the Shah reference teaches a method of detecting a target nucleic acid by hybridizing a target to a capture probe and detector probe and detecting the bound hybrid and further use of bridge probes to limit background noise in hybridization assays. Applicants respectfully disagree with the Examiner's grounds for rejection.

The Shah reference describes a method of capture-release, where a target is hybridized with a first capture probe and a detector probe at two different regions of the target molecule, where the capture probe is used to bind the resulting complex to a solid support. After target capture, the target is released from the first solid support under conditions where the first capture probe remains bound to the solid surface since it has a higher affinity for the solid support than the target, and the labeled detector probe remains bound to the target. The target- detector probe complex is then exposed to a second capture probe which forms a new complex with the target (see col. 3, ln. 65 – col. 4, ln.10; emphasis added). This function requires that the second capture probe be complementary to the target sequence and the detector probe be labeled with a detectable moiety (col. 1, lns. 30-32). The Examiner equates "detector probe" to the signal sequence probe (SSP) of the instant invention. However, the claimed SSP is not detectably labeled and applicants have amended, herewith, claim 1

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(depending claims 6-9) to indicate that the SSP is unlabeled for clarity's sake. The Shah method uses multiple capture-release steps to significantly decrease the background noise, even to the point of completely eliminating background (col. 3, lns. 44-48), whereas the instant specification uses blocker probes to reduce background. There is no motivation in Shah to change Shah's method and use the blocker probes of Coull in order to reduce background. Because the capture-release method of Shah eliminates background noise almost completely, the skilled artisan would not be motivated to substitute Coull's method.

The Shah reference at col. 8, lns. 58-67, as cited by the Examiner, relates to the use of "dA-tailed probes." The Examiner equates these to the bridge probes of the present invention. The Examiner further contends that the bridge probes are used to decrease background noise (Paper No. 18, pg. 7, lns. 16-17). Applicants respectfully disagree with this interpretation of the Shah reference.

The use of bridge probes in Shah is not used as taught in the instant invention. It is clear that these dA-tailed probes in Shah are not acting as bridge probes, *i.e.* hybridizing to target and the signal sequence probe (instant specification, pg. 7, ln. 9- pg. 8, ln. 2), but rather "such dA-tailed probes will bind to both target and dT derivatized supports" (Shah; col. 8, lns. 48-50). The dA-tailed probes act as a bridge between the solid support and the target, but not as a means for serving as "an intermediate for connecting the SSP to the target nucleic acid and the CSP hybridized to the target nucleic acid" (instant specification, pg. 7, lns. 18-19). The dA-tailed probes of Shah and the bridge probes of the claimed invention function differently and serve different purposes. The Shah dA-tailed probes capture the target to solid supports, whereas the bridge probes of the present invention enable signal sequence probes to complex with the target already bound to support. Therefore, Shah, as cited by the Examiner, does not teach or suggest bridge probes as claimed. Neither do the bridge probes serve to reduce signal to noise ratio in hybridization assays. The Examiner has cited Col. 8, lns. 15-42 of Shah to point out that the bridge probes serve to lessen background signal to noise ratio. However, there is no mention of bridge probes in this particular section. Even in the bridge probe section (col. 8, lns. 43-54) there is no mention that bridge probes lessen background signal. Applicants respectfully direct the Examiner's attention to col. 7, lns. 30-41, where the capture-release method is used to remove excess second capture probe from

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non-specifically binding to the first support which may cause noise, and thereby decrease background.

As the Examiner surely knows, dependent claims cannot be obvious in view of the art if the art does not render the broader independent claim obvious. Thus, regardless of whether Shah describes dA-tailed probes, biotinylated capture probes, or capture probes and detector probes having a distance of less than 3 kb when hybridized to target, claims 6-8, 45, 49, 52, and 54-55 are not obvious because Coull does not disclose the independently claimed method of detecting target nucleic acid. Nor is there any motivation to combine the method of Coull with Shah since the capture-release method of Shah almost completely eliminates background noise.

The combination of Coull and Shah fails to make the present invention obvious. Specifically, Coull fails to teach or suggest an assay using three different types of probes as claimed, where two probes hybridize to the target nucleic acid and the third blocker probe binds to excess capture probe (claims 1-2, dependent claims 6-9; claims 50-55). Specifically, Coull, et al. fails to motivate one skilled in the art to modify the teachings of Coull to result in blocker probes having sequences identical to the target for hybridizing to excess non-hybridized capture sequence probes. Shah, et al. does not remedy this deficiency.

Further, Shah also does not teach or suggest bridge probes because the analogous probe (i.e., the dA-tailed probe) has no affinity for the signal sequence probe, which is a required function of the bridge probes of the present invention. Therefore, none of the references, alone or when viewed in combination, teach or suggest the blocker or bridge probes of the claimed method or the claimed method as a whole. For further clarity, applicants have amended claim 1 to indicate that the signal sequence probe is unlabeled. Thus, in reading the cited references, the skilled artisan would not be able to generate the claimed method, which uses blocker probes for preventing non-specific hybridization of capture oligonucleotides to a target or bridge probes for hybridizing target and signal sequence probes for detection. None of the references, even when viewed in combination teach or suggest the use of these blocker or bridge probes. Thus, applicants respectfully request reconsideration and withdrawal of the §103(a) rejection.

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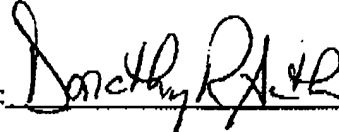
Allowance of the pending claims is respectfully requested. Early and favorable action by the Examiner is earnestly solicited.

As required by 37 C.F.R. §1.121, a marked up version of the replacement claims is provided and attached hereto, where additions and deletions are indicated by underlining and bracketing.

The Commissioner is hereby authorized to charge any additional fees which may be required for the timely consideration of this amendment under 37 C.F.R. §§ 1.16 and 1.17, or credit any overpayment to Deposit Account No. 13-4500, Order No. 2629-4017. A
DUPLICATE COPY OF THIS SHEET IS ATTACHED.

Respectfully submitted,

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Dated: January 17, 2003

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VERSION WITH MARKINGS TO SHOW CHANGES MADE**IN THE CLAIMS**

Please amend the claims as follows:

1. (amended) A method of detecting a target nucleic acid comprising:
 - a) hybridizing a single stranded target nucleic acid to a capture sequence probe and a signal sequence probe to form double-stranded hybrids between said probes and the target nucleic acid, wherein the capture sequence probe and the signal sequence probe are capable of hybridizing to non-overlapping regions within the target nucleic acid and not being capable of hybridizing to each other, wherein the signal sequence probe is unlabeled and said hybridization forms a sequence probe:target hybrid; and
 - b) adding a blocker probe to the hybridization reaction, wherein said blocker probe hybridizes to excess non-hybridized capture sequence probes;
 - c) capturing the sequence probe:target hybrid to form a bound hybrid; and
 - d) detecting the bound hybrid.
37. (amended) The method of claim 20 [22], further comprising adding a blocker probe to the hybridization step, wherein said blocker probe hybridizes to excess non-hybridized capture sequence probes.

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